

Plasticity in functional traits in the context of climate change: a case study of the subalpine forb *Boechera stricta* (Brassicaceae)

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Abstract

Environmental variation often induces shifts in functional traits, yet we know little about whether plasticity will reduce extinction risks under climate change. As climate change proceeds, phenotypic plasticity could enable species with limited dispersal capacity to persist *in situ*, and migrating populations of other species to establish in new sites at higher elevations or latitudes. Alternatively, climate change could induce maladaptive plasticity, reducing fitness, and potentially stalling adaptation and migration. Here, we quantified plasticity in life history, foliar morphology, and ecophysiology in *Boechera stricta* (Brassicaceae), a perennial forb native to the Rocky Mountains. In this region, warming winters are reducing snowpack and warming springs are advancing the timing of snow melt. We hypothesized that traits that were historically advantageous in hot and dry, low-elevation locations will be favored at higher elevation sites due to climate change. To test this hypothesis, we quantified trait variation in natural populations across an elevational gradient. We then estimated plasticity and genetic variation in common gardens at two elevations. Finally, we tested whether climatic manipulations induce plasticity, with the prediction that plants exposed to early snow removal would resemble individuals from lower elevation populations. In natural populations, foliar morphology and ecophysiology varied with elevation in the predicted directions. In the common gardens, trait plasticity was generally concordant with phenotypic clines from the natural populations. Experimental snow removal advanced flowering phenology by 7 days, which is similar in magnitude to flowering time shifts over 2–3 decades of climate change. Therefore, snow manipulations in this system can be used to predict eco-evolutionary responses to global change. Snow removal also altered foliar morphology, but in unexpected ways. Extensive plasticity could buffer against immediate fitness declines due to changing climates.

Keywords: *Boechera stricta*, ecophysiology, elevation gradient, flowering phenology, functional traits, phenotypic plasticity, snow removal experiment, snow melt, subalpine meadow

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Introduction

Climatic factors strongly influence species' distribution patterns and evolutionary trajectories (Etterson, 2004; Chen *et al.*, 2011; Kim & Donohue, 2013). Contemporary climate change has caused some species to shift their distributions, primarily via range contractions at the warmer margin, sometimes accompanied by expansions into historically cooler habitats at higher elevations and poleward latitudes (e.g. Parmesan & Yohe, 2003; Hickling *et al.*, 2006; Kelly & Goulden, 2008; Lenoir *et al.*, 2008). Yet, not all species are altering their

distributions in the predicted fashion, perhaps because temperature is not the only determinant of range limits (Lenoir *et al.*, 2010; Zhu *et al.*, 2012; Macias-Fauria & Johnson, 2013; Harsch & Hille Ris Lambers, 2014), and because phenotypic plasticity and adaptive evolution can theoretically enable population persistence *in situ* (Chevin & Lande, 2010; Chevin *et al.*, 2010; Nicotra *et al.*, 2010; Vedder *et al.*, 2013).

Models typically assume that species respond to climate change primarily via distributional shifts, and generally neglect evolutionary processes and phenotypic plasticity (but see Chevin *et al.*, 2010; Kuparinen *et al.*, 2010; Norberg *et al.*, 2012). Adaptive phenotypic plasticity can evolve when abiotic and biotic conditions vary reliably through space and time, and individuals experience multiple environments throughout their lifetimes, or progeny inhabit different environments

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than the parents (e.g. Moran, 1992; Stratton & Bennington, 1998; Alpert & Simms, 2002; Van Kleunen & Fischer, 2005; Baythavong, 2011). Although individuals of numerous species can adjust functional phenotypes plastically to cope with spatial and temporal heterogeneity in conditions (e.g. Donohue *et al.*, 2000; Anderson *et al.*, 2010; Baythavong & Stanton, 2010; Torres-Dowdall *et al.*, 2012), plasticity is typically overlooked in global change studies (Nicotra *et al.*, 2010).

In the context of global change, increased temporal variation in climatic conditions could favor plasticity (Chevin *et al.*, 2010; Nicotra *et al.*, 2010). In addition, plasticity could enable migrating populations to establish under new biotic and abiotic conditions in their expanded geographic ranges. Longitudinal studies have uncovered extensive plasticity to changing climates in animal and plant systems (e.g. Teplitsky *et al.*, 2008; Morin *et al.*, 2009; Ozgul *et al.*, 2009; Amano *et al.*, 2010; Gordo & Sanz, 2010; Moyes *et al.*, 2011; Anderson *et al.*, 2012; Vedder *et al.*, 2013). In most cases, however, we do not know if plasticity in phenology and morphology confers a fitness advantage, or is a maladaptive response to resource limitation or other stresses. Climate change could induce adaptive plasticity, exposing novel phenotypes that allow populations to persist in their current locations or establish in new sites (Nicotra *et al.*, 2010). Alternatively, climate change could induce maladaptive trait shifts, depressing fitness, and potentially increasing the risk of local extinctions (Ghalambor *et al.*, 2007). Will plasticity in ecologically relevant traits buffer populations against extinction in the short term (Chevin *et al.*, 2010)?

In many ecosystems at high elevations and mid to high latitudes, warming winter temperatures are reducing snowpack depth, and elevated spring temperatures are causing the remaining snow to melt earlier than during preindustrial times (Stewart, 2009; Clow, 2010; Pederson *et al.*, 2011). Plant communities in these systems rely on snow melt for irrigation, as most precipitation falls during the winter months as snow (Dunne *et al.*, 2003). A shift from snow to rain in the winter months reduces water availability during the dry growing season, and earlier snow melt lengthens the time between snow melt and summer monsoons, increasing the probability of water stress (Woodhouse, 2003; Rangwala *et al.*, 2012). Snow melt signals the beginning of spring, and influences life history transitions, such as emergence from hibernation and the initiation of reproduction (Dunne *et al.*, 2003; Lane *et al.*, 2012; Leingärtner *et al.*, 2014). Early snow melt can expose communities to frosts that they would not have experienced historically when they were still insulated by a blanket of snow (Inouye, 2008).

In subalpine meadows of Colorado, *Boechera stricta* (Brassicaceae) now blooms more than 2 weeks earlier in the growing season than it did in the mid-1970s (Anderson *et al.*, 2012; Caradonna *et al.*, 2014). Flowering phenology is positively correlated with the timing of snow melt from 1973 to 2012 for *B. stricta* (Anderson *et al.*, 2012). We hypothesize that reductions in snowpack and concomitant advancements in snow melt induce earlier flowering. Here, we use an experimental framework to test the causal relationship between snowbank dynamics and flowering phenology and examine plasticity in functional traits.

In our system, temperatures decline, snow melts later and soil moisture increases with elevation (Dunne *et al.*, 2003). As the climate continues to warm, we expect high elevation populations to be exposed to warmer and drier conditions that reflect contemporary climates at lower elevations. Thus, we hypothesize that traits that promote survival and reproduction at low elevations should, in future climates, be advantageous at higher elevation locations. We focus our study on phenological, morphological, and ecophysiological traits have been implicated in adaptation to climate (e.g. Campbell *et al.*, 2010; Nicotra *et al.*, 2011; Leonardi *et al.*, 2012; Ward *et al.*, 2012; Lee & Mitchell-Olds, 2013; Pratt & Mooney, 2013): flowering phenology, specific leaf area, stomatal anatomy, foliar N content (%N), natural abundance stable carbon and nitrogen isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively), and leaf water content. We leverage data from our previously published record of long-term shifts in flowering phenology (Anderson *et al.*, 2012), clinal variation in functional traits from natural populations (this study), and patterns of plasticity in common gardens (this study) to develop predictions of how climate change could alter trait expression (Table 1). We then test these predictions by manipulating snow dynamics to assess climate-mediated plasticity.

Materials and methods

Study site and focal species

Boechera stricta is a primarily self-pollinating forb native to the Rocky Mountains, where it is commonly found across a broad elevational gradient (1500–3700 m) (Song *et al.*, 2006; Rushworth *et al.*, 2011). Studies in contemporary environments have documented local adaptation to latitude (Anderson *et al.*, 2014a) and water availability (Lee & Mitchell-Olds, 2013). The center of genetic diversity for *B. stricta* is in the Colorado Rocky Mountains (Kiefer *et al.*, 2009); therefore, our study sites around the Rocky Mountain Biological Laboratory (RMBL; Gothic, Gunnison county, Colorado) are in the portion

Table 1 Predictions of how traits could vary with elevation and snow removal treatment. Positive and negative symbols show the direction of the relationship between a trait and elevation (e.g. + indicates a positive relationship between trait values and elevation)

Trait	Elevation	Snow removal induces
Flowering phenology	–*	Accelerated flowering
Specific Leaf Area	–	Lower SLA (thicker leaves)
Leaf water content	+	Lower trait values
Stomatal density	+	Reduced stomatal density
Stomatal size	–	Larger stomata†
Water-use efficiency	–	Greater WUE (more positive $\delta^{13}\text{C}$ values)
Foliar N content (%N)	+	No change
Stable N isotopes ($\delta^{15}\text{N}$)	+	No change

*In natural populations, snow melt occurs later at high-elevation sites; therefore, plants initiate flowering at a later calendar date (positive relationship between flowering phenology and elevation). In the short growing seasons of high-elevation sites, we expect adaptations that allow for rapid development and reproduction. For that reason, we predict that high-elevation genotypes will flower earliest in common gardens (negative relationship between flowering phenology and source elevation of a genotype). We quantified flowering phenology in the experimental gardens and snow removal experiment, not in natural populations.

†This prediction of larger stomata derives primarily from the inverse relationship between stomatal density and stomatal size.

of the range where within-population genetic variation could potentially enable continued adaptation to progressively new climates.

Trait predictions

Foliar, ecophysiological, and phenological traits are highly sensitive to climatic conditions (Medeiros & Ward, 2013; Pratt & Mooney, 2013). For example, many species of plants exhibit phenotypic plasticity in specific leaf area (SLA: leaf area per unit dry mass): low light, ample soil water availability (in the absence of waterlogging), high temperatures, low atmospheric CO_2 concentrations, and to a lesser extent high soil nutrient levels generally induce thin leaves with low tissue density (high SLA) (Ainsworth & Long, 2005; Poorter *et al.*, 2009; Scheepens *et al.*, 2010). We predict that SLA will decline with elevation because of lower temperatures at higher elevations (e.g. Scheepens *et al.*, 2010), and that water stress associated with snow removal will induce thicker leaves (lower SLA; Table 1).

Stomata regulate gas exchange between the leaf and the atmosphere. Maximum stomatal conductance is determined by anatomical traits, including stomatal density and size (Dow

et al., 2014). Under drought stress, stomatal density decreases to prevent water loss via transpiration (Woodward *et al.*, 2002). Stomatal anatomy varies with other climatic factors, including atmospheric (CO_2), air temperature, relative humidity, and light level (Casson & Gray, 2008; Lake & Woodward, 2008; Franks *et al.*, 2013). We predict that stomatal density will increase with elevation (Woodward *et al.*, 2002) and decrease with snow removal. Owing to a negative correlation between stomatal density and size (e.g. Franks *et al.*, 2013), we expect stomata to be smaller at higher elevations.

We predict that enhanced water-use efficiency will be favored in dry, low-elevation populations and under climate change. Stable Carbon isotope values ($\delta^{13}\text{C}$) reflect the ratio of CO_2 concentration inside the leaf to atmospheric [CO_2] (c_i/c_a), which is generally correlated with water-use efficiency integrated over the life span of the leaf (Farquhar *et al.*, 1989). Foliar %N is a good indicator of photosynthetic rate as most leaf N occurs in photosynthetic proteins (Evans, 1989). As rapid photosynthesis should be advantageous in the abbreviated summers of high elevation, we predict that foliar %N will increase with elevation. Foliar natural abundance stable N isotope ratios ($\delta^{15}\text{N}$) reflect ecosystem-level N cycling and environmental conditions, and are generally greatest in hot and dry climates (Craine *et al.*, 2009), such as our low-elevation sites. Thus, $\delta^{13}\text{C}$ and foliar %N provide insights into plant ecophysiology, while $\delta^{15}\text{N}$ levels provide information about the climate (Craine *et al.*, 2009).

Foliar trait variation across elevational gradient

To examine clinal variation in foliar traits along natural climatic gradients, we quantified SLA, stomatal density and size, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and foliar %N in *B. stricta* populations in rocky subalpine meadows sampled across four mountain valleys near the RMBL in 2012 and 2013 (elevations: 2881 m–3629 m). In these valleys, we systematically searched hiking trails and collected from natural populations that were at least 50 m off the trail. We located sites with robust endogenous natural populations of *B. stricta* that were 20–40 m in radius and separated from each other by at least 150 m. We sampled three additional sites 7.7–25.4 km south to include natural populations at lower elevations (2543–2766 m) than present in the four valleys (see Table S1 for elevations, GPS coordinates, sample sizes per population, and interpolated climatic data from PRISM databases). Within populations, we collected leaves from individuals that were >2 m apart to reduce the probability of sampling siblings. To avoid duplicate collections per individual of this perennial species, we did not sample from the same population in both years (in total: $N = 601$ plants in $N = 45$ unique populations over 2 years). Instead, we collected leaves from different populations on the same mountain across years.

We retrieved interpolated climatic data for all sites from PRISM databases (accessed via <http://www.cefa.dri.edu/Westmap/westmappass.php> on September 2, 2014), which include high elevation sites from the Snowpack Telemetry Network (snotel). The PRISM database contains monthly precipitation and temperature data from 1895 to present with a

spatial resolution of 2.5 arc-min (~4 km). In the subset of the populations that were separated by >4 km ($N = 15$ sites), mean annual air temperature declines by 0.0023 °C for every 1 m gain in elevation ($F_{1,13} = 12.7$, $P = 0.0034$, $r^2 = 0.49$), and total annual precipitation increases by 0.042 cm/m ($F_{1,13} = 22.05$, $P = 0.0004$, $r^2 = 0.63$). To achieve a more precise estimate of the relationship between elevation and temperature, we accessed 2010 and 2011 climatic data from six weather stations near our sites (data retrieved from <http://rmb.org/> on July 24, 2013). Mean annual temperature (MAT) declined by 0.0046 °C (± 0.0012) per 1 m elevation gain ($F_{1,4} = 14.2$, $P = 0.0197$; local weather stations at elevations: 2469, 2860, 2917, 3003, 3396, and 3421 m).

In 2012, we collected 3–5 rosette or cauline leaves of 278 individuals from 16 populations (elevations: 2734–3480 m). Rosette leaves are produced early in the season when water availability is high due to snow melt. Cauline leaves develop several weeks later, only on plants that successfully bolt prior to reproduction. These two leaf types experience different light and soil moisture conditions during development, and we expect drought stress to be more pronounced for cauline leaves. In 2012, we collected leaves in July when some bolting plants had already senesced their rosette leaves; we sampled cauline leaves if rosette leaves were not available. In 2013, we completed leaf collection earlier in the season to sample rosette leaves for all plants (elevations: 2543–3629 m; $N = 29$ populations; $N = 323$ individuals).

We scanned fresh leaves to calculate leaf area using Image J (Schneider *et al.*, 2012), dried all leaves in an oven at 50 °C for 3 days, and then weighed dried samples. We calculated specific leaf area (SLA) as leaf area/dried weight. We used clear nail polish to create epidermal impressions of the abaxial leaf surface, which we mounted on microscope slides to examine stomata (e.g. Xu & Zhou, 2008). In 2012, we prepared epidermal impressions from dried leaves, whereas in 2013, we only used fresh leaves collected the same day. We sampled 528 individuals for stomatal anatomy. We photographed four distinct nonoverlapping 0.0352 mm² areas of each impression under 400 × magnification using a compound microscope at the University of South Carolina, and, averaged the stomatal density over the four fields of view. We then used ImageJ to quantify stomatal size (length of the stomatal pore) for six stomata per plant by measuring the distance between the two inner junctions of the guard cells (Zhang *et al.*, 2012).

To quantify ecophysiological traits, we ground leaves using a GenoGrinder (SPEX SamplePrep, Metuchen, NJ, USA), then weighed 3.000–3.200 mg of foliar tissue on a ultramicrobalance (UMX2, Mettler-Toledo, Columbus, OH, USA) and loaded tissue into tin capsules (CE Elantech, Lakewood, NJ, USA). These samples were combusted on an isotope ratio mass spectrometer connected to an elemental analyzer at the Cornell University Stable Isotope Lab. As is standard, we report $\delta^{13}\text{C}$ relative to Vienna Pee Dee Belemnite and $\delta^{15}\text{N}$ relative to atmospheric N₂. We analyzed foliar tissue from $N = 5.12 \pm 0.9$ (mean \pm SD) individuals per natural population ($N = 220$ individuals

total) for $\delta^{13}\text{C}$, foliar N content (%N on a per mass basis), and $\delta^{15}\text{N}$. Of these samples, 49 were from cauline leaves and 171 were from rosette leaves.

We conducted a multivariate mixed model in Proc Mixed (SAS ver. 9.3) to test whether foliar trait values varied with elevation (a proxy for climate), leaf type (rosette vs. cauline) and year, with a random effect for population nested within mountain. We did not include interaction terms between leaf type and elevation or elevation and year, as preliminary analyses indicated that these interactions were nonsignificant. Results were qualitatively and quantitatively similar when analyses were restricted to rosette leaves (not shown).

We included the following foliar traits as response variables: stomatal density, stomatal size, SLA, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and foliar N content. We standardized all traits to a mean of 0 and a standard deviation of 1 to enable convergence of the multivariate model because traits were measured on different scales. As the multivariate model was highly significant, we conducted univariate analyses to examine the effect of elevation on each trait, using a Bonferroni corrected alpha to determine significance in the tests of six foliar traits ($\alpha = 0.05/6 = 0.00833$). We also calculated pairwise trait correlations (Proc Corr).

Common garden experiment: heritability and trait variation

Clinal variation in traits can result from genetic differentiation among populations and environmental divergence across sites. Common garden or reciprocal transplant experiments are powerful approaches that disentangle the contributions of environment (plasticity) and evolutionary history (genotype and genotype by environment interactions) to trait expression by exposing siblings to disparate conditions (Anderson *et al.*, 2014b). Furthermore, quantitative genetic field experiments are necessary to estimate heritability in traits and plasticity, and to assess whether populations maintain sufficient genetic variation to enable them to respond to novel selection mediated by climate change. For those reasons, we installed a common garden experiment at two elevations (2891 and 3133 m; coordinates and PRISM climatic data are available in Table S1) to examine plasticity and heritability in phenology and foliar morphology at a regional scale. We do not have climatic data from these gardens for 2013, but in 2014, we installed 5TM Decagon soil moisture and temperature sensors in 5 blocks/garden. From July 2, 2014–August 28, 2014, the low-elevation garden was an average of 4.52 °C \pm 0.04 (mean \pm S.E.) warmer than the high-elevation garden ($F_{1,8} = 12583.5$, $P < 0.0001$, Anderson, unpublished data).

We included one maternal family from each of $N = 24$ populations at elevations ranging 2869–3682 m (see Table S2 for coordinates and source elevations of the 24 families). We grew field-collected seeds for one generation in the greenhouse to reduce maternal effects and produce self-fertilized families of this inbred species.

In September 2011, we transplanted $N = 2293$ juvenile rosettes (~95.5 individuals per family) into the lower elevation garden only. In September 2012, we planted another

set of rosettes into both gardens ($N = 1134$ individuals in the lower garden and $N = 1128$ individuals in the higher garden from the same 24 families as in 2011). In both years, we planted two siblings per maternal family into blocks of 48 individuals within the matrix of natural vegetation. Fencing around each garden excludes cows (present in the fall) and gophers, whose winter tunneling and foraging can damage plants.

From May to August 2013, we visited each garden 3–4 times/week and recorded survivorship, plant size, flowering, and fruiting. In June 2013, we sampled 1–10 fully mature leaves per individual (mean \pm SD: 3.38 ± 1.51 leaves/plant) from $N = 438$ transplants at the high-elevation garden and $N = 912$ transplants at the low-elevation garden ($N = 545$ from the 2011 cohort and 367 from the 2012 cohort). We could not collect leaves from plants that had died or had no mature leaves. We quantified SLA and leaf water content [(fresh weight – dried weight)/fresh weight] for all collections, stomatal anatomy for 6.2 ± 3.9 (mean \pm SD) siblings per family ($N = 416$ plants total), and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and foliar N content for 2.98 ± 0.5 (mean \pm SD) siblings per family per garden for the 2012 cohort only ($N = 142$ total).

Broad-sense heritability. In self-pollinating species, such as *B. stricta* (Song *et al.*, 2006), selection operates on total genetic variance, not just additive genetic variance (Roughgarden, 1979). Therefore, we used the restricted maximum likelihood (REML) method (Proc Mixed) to estimate broad-sense heritability as total genetic variance (V_G) divided by phenotypic variance ($V_P = \text{family variance} + \text{block variance} + \text{error variance}$) for life history and foliar traits in models that included a fixed effect for cohort (low elevation only) and random effects for genotype and block. In addition, we estimated heritability in plasticity for all traits as genetic variation in genotype by environment interactions ($V_{G \times E}$) divided by phenotypic variation (V_P) in models that included fixed effects for environment (garden) and cohort, and random effects for genotype, genotype \times environment, and block nested within garden (Scheiner & Lyman, 1989). These heritability estimates do not reflect within-population genetic variation because we sampled only one family per population. Rather, they place upper bounds on the extent of genetic variation in this regional pool of families.

Clinal trait variation. We tested whether clinal variation in traits is driven by a plastic response to divergent environments or whether genetic variation also contributes to phenotypic variation. In the first case (plasticity), we expect trait expression to differ in the two common gardens, but no relationship between trait values and the elevation of origin of the family. In the second case, we expect that the relationships between trait values in the gardens and provenance elevation of the 24 families mirror clinal variation detected in natural populations. We modeled traits as a function of garden environment, elevation of origin of the families, and cohort in mixed models with random effects for family (i.e. genotype), family crossed with garden (i.e. genotype by environment), and block nested in garden.

Owing to differences in sample sizes per trait, and different distribution patterns, we ran a series of univariate analyses for 10 traits: leaf water content (log transformed), flowering time, duration of flowering, height at flowering, specific leaf area, stomatal density, stomatal pore length, $\delta^{13}\text{C}$, foliar %N, and $\delta^{15}\text{N}$. We analyzed all traits with mixed models (Proc Mixed), except for flowering phenology for which we ran discrete-time survivorship analyses (Cox proportional hazards models). Random effects (frailty) modeling permits the analysis of clustered survivorship data, which arise when time-to-event data are collected on family members (Kelly, 2004). Unlike Proc Phreg (SAS ver. 9.3), the R package coxme (ver. 2.2–3, Therneau, 2012) allows for multiple random effects, including nested effects, in Cox proportional hazards models (frailty models). However, as coxme does not currently permit crossed random effects, our flowering phenology models included only family and block nested within family as random effects. We ran a complementary generalized linear mixed model in Proc Glimmix (SAS, ver. 9.3) using a Poisson distribution with a log link for time at first flowering and general linear mixed model (Proc Mixed) for flower duration to examine the full models (including the random effect of genotype crossed with environment). To assess significance across all analyses, we used a Bonferroni corrected alpha for 10 traits ($\alpha = 0.005$).

Experimental climate manipulation

To investigate whether reduced snowpack and early snow melt accelerate flowering and alter foliar morphology in ways concordant with predictions (outlined in Table 1), we conducted a snow removal experiment in three subalpine meadows near the RMBL from 2011 to 2013. We selected these sites based on habitat and accessibility for snow removals during the late winter. The three sites were similar in elevation, ranging 2997–3004 m, and the two farthest sites were <230 m from each other (see Table S1 for coordinates). At each site, we established a series of $2 \times 2 \text{ m}^2$ plots, randomly assigned to one of two treatments: snow removal vs. control (see Figures S1–S3 for maps of each site). To set out plots, Z. Gezon dropped flags approximately every 5 m while skiing across each meadow in late winter. After establishing plots, Gezon randomly assigned them to either snow removal or control treatments using a random number generator. Within each site, plots did not differ significantly in terms of elevation, aspect, plant species composition, and environmental conditions (results not shown). *Boechea stricta* was present initially, but plot placement was randomized and not based on the abundance or presence of *B. stricta*. Of the 30 plots established in 2011, 17 had *B. stricta* individuals.

When snowpack receded to ~1 m in depth in April or May, we shoveled snow to 5 cm in the snow removal plots and in buffer zones 0.25 m on every side of the plots (similar to Dunne *et al.*, 2003). Snow depth at time of shoveling in April 2012 was 40 cm due to extremely low snowfall in that year. Control plots experienced natural snow melt.

We expanded the sample size over the course of the study ($N = 15$ plots per treatment in 2011; 18 plots per treatment in

2012; 20 removal and 21 control plots in 2013). The exact location of the plots remained the same at one site (Marriage Meadow) throughout the duration of the experiment, but changed at the other sites. As this study lasted only 3 years, and *B. stricta* is a perennial with a generation time of 2–3 years (Anderson *et al.*, 2012), this experiment tests for short-term shifts in traits that are likely the result of phenotypic plasticity, not adaptation to changing conditions.

Snow removal experiment: phenology. During the growing season, we visited each plot every other day to record snow depth, percent bare ground, and the timing of flowering of *B. stricta* individuals that recruited naturally into the plots. In 2011, we collected phenology data from all three sites, but in 2012 and 2013, our phenological measurements were limited to one site (Marriage Meadow), although manipulations occurred at all three sites. We encountered reproductive *B. stricta* individuals in only a subset of the plots (2011: 23 individuals in 10 removal plots and 18 individuals in 7 control plots; 2012: 3 individuals in 2 removal plots and 5 individuals in 4 controls; 2013: 8 individuals in 2 removal plots and 6 individuals in 4 control plots).

Analyses. We examined the efficacy of the manipulations through a repeated measures analysis of the timing of snow melt (Julian day on which a plot was 100% free of snow) as a function of treatment, year, treatment by year, and site, with a repeated statement for year (autoregressive correlation structure). We then modeled flowering phenology as a function of treatment, year, treatment by year, and site in a repeated measures ANOVA. As the residuals were slightly heteroskedastic, we used an unequal variance model with Satterthwaite approximation for degrees of freedom (Proc Mixed). Flowering phenology was a plot level variable: the Julian day on which the first *B. stricta* flower was observed in a plot represented the timing of first flowering, and the day when the greatest number of *B. stricta* flowers were present represented the timing of peak flowering (see also, Anderson *et al.*, 2012). In a given year, each plot had one data point for first flowering and one for peak flowering. We found very similar results in complementary analyses restricted to data: (i) from 2011 when phenology was monitored at all three sites (Table S3a) and (ii) from the Marriage Meadow site only, where we studied phenology from 2011 to 2013 (Table S3b).

For comparison with the previous correlation between snow melt and first flowering in a 38-year dataset (Anderson *et al.*, 2012), we regressed the timing of first flowering on snow melt timing in a repeated measures analysis with fixed effects for site and year, again using an unequal variance model (Proc Mixed).

Snow removal experiment: foliar traits. We predicted that plants exposed to early snow removal would resemble individuals from lower elevation populations. We inspected all plots in all three sites thoroughly for juvenile and reproductive *B. stricta* plants in July 2012 and 2013. We collected 3–5 rosette (juveniles) or cauline (adults) leaves per individual. One site had only five *B. stricta* in 2012 ($N = 4$ in control and 1

in removal plots) and no *B. stricta* plants in 2013. We removed that site from the analysis, but including it produced qualitatively similar results (not shown). We sampled a total of $N = 90$ individuals (2012: $N = 9$ adults in control and 8 adults in removal plots; 2013: $N = 22$ adults and 22 juveniles in control and 13 adults and 16 juveniles in removal plots). We processed samples as described for the natural populations. In 2013, we stored leaves on ice, immediately brought them to the laboratory and recorded fresh weight within 3–4 hours of collection. For the 2013 samples, we calculated leaf water content [(fresh weight – dried weight)/fresh weight], but we do not have fresh weights for the 2012 samples.

We conducted a multivariate regression to test whether foliar traits vary plastically as a function of treatment in each plot. Response variables were modeled simultaneously, and included: leaf water content (log transformed), specific leaf area, stomatal density, and stomatal pore length. We did not analyze $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and foliar %N because half of the samples from this experiment were destroyed through failure of the mass spectrometer, leaving us with insufficient sample sizes. We modeled fixed effects for treatment, leaf type (rosette vs. cauline), site, year, and leaf type by treatment on multivariate foliar traits. We included block nested within site as a random effect. As the multivariate analysis generated significant results, we did follow-up univariate analyses, with a Bonferroni corrected alpha for tests of four traits ($\alpha = 0.05/4 = 0.0125$).

Results

Foliar trait variation across elevational gradient

We found significant effects of elevation ($F_{6,2040} = 8.7$, $P < 0.0001$), year, ($F_{6,2040} = 18.9$, $P < 0.0001$) and leaf type ($F_{6,2040} = 56.9$, $P < 0.0001$) on the multivariate suite of foliar traits. In univariate analyses, after Bonferroni correction, the following traits varied in the expected direction with elevation: stomatal density, foliar N content, and $\delta^{15}\text{N}$ (Table 2; Fig. 1). Counter to expectations, specific leaf area increased with elevation. There was no relationship between elevation and stomatal size or $\delta^{13}\text{C}$ (Table 2). In pairwise phenotypic correlations, we found the expected negative relationship between stomatal density and size (Pearson's correlation coefficient = -0.48 , $P < 0.0001$, $N = 518$), but failed to detect a positive correlation between foliar N and $\delta^{15}\text{N}$ ($P = 0.90$; Table S4). Specific leaf area was positively correlated with stomatal density ($r = 0.13$, $P = 0.0032$, $N = 522$) and negatively correlated with stomatal size ($r = -0.28$, $P < 0.0001$, $N = 518$) and $\delta^{13}\text{C}$ ($r = -0.42$, $P < 0.0001$, $N = 220$); water-use efficiency is often positively correlated with $\delta^{13}\text{C}$), suggesting that thin leaves had high stomatal density, small stomata, and limited water-use efficiency (Table S4).

Table 2 Clinal variation in foliar traits across elevation in natural populations of *Boechera stricta*. These results are based on separate mixed model analyses for each trait. We present raw *P*-values, but have highlighted in bold the predictors that are significant after Bonferroni correction of six traits ($\alpha = 0.05/6 = 0.00833$)

Source	Specific leaf area (cm ² /g) (Log transformed)		Stomatal density (stomata/mm ²)		Stomatal pore length (µm)		δ ¹³ C		%N		δ ¹⁵ N	
	<i>F</i> _{1,551}	<i>P</i>	<i>F</i> _{1,482}	<i>P</i>	<i>F</i> _{1,478}	<i>P</i>	<i>F</i> _{1,175}	<i>P</i>	<i>F</i> _{1,175}	<i>P</i>	<i>F</i> _{1,175}	<i>P</i>
Elevation	7.16	0.0077	9.42	0.0023	3.58	0.059	1.24	0.27	10.72	0.0013	19.68	<0.0001
Leaf type	285.17	<0.0001	2.86	0.0913	35.5	<0.0001	0.58	0.25	0.01	0.91	0.28	0.6
Year	7.35	0.0069	57.52	<0.0001	48.11	<0.0001	1.28	0.26	2.36	0.13	3.85	0.052
Population (mountain)	$\chi^2 = 197.1$	<0.0001	$\chi^2 = 70.2$	<0.0001	$\chi^2 = 42.1$	<0.0001	$\chi^2 = 23.7$	<0.0001	$\chi^2 = 5.2$	0.0225	$\chi^2 = 57.6$	<0.0001

Common garden experiment: heritability and trait variation

Heritability. Most traits had significant broad-sense heritability: flowering phenology, duration of flowering, plant size at flowering, and specific leaf area in both gardens, stomatal density in the lower elevation garden, and leaf water content and δ¹³C in the higher garden (Table 3). We found no evidence for heritability in stomatal size, foliar N content, or δ¹⁵N in either garden. However, there was a negative genetic correlation between stomatal density and size ($r_G = -0.98 \pm 0.27$, $\chi^2 = 19.6$, $P < 0.0001$). Finally, three traits showed significant heritability in plasticity: height at flowering, specific leaf area, and leaf water content.

Trait variation. After Bonferroni correction, six traits had significant plasticity in response to common garden environment: flowering time ($z = -23.1$, $P < 0.0001$), specific leaf area ($F_{1,22} = 115.8$, $P < 0.0001$), stomatal density ($F_{1,19} = 46.3$, $P < 0.0001$), stomatal size ($F_{1,20} = 72.4$, $P < 0.0001$), foliar %N ($F_{1,22} = 21.5$, $P = 0.0001$), and δ¹⁵N ($F_{1,22} = 183.1$, $P < 0.0001$; Table S5; Fig. 1). For flowering phenology, results of complementary analyses (Cox proportional hazards frailty models and generalized linear mixed models) were very similar (Table S5). Plasticity was generally concordant with trait variation from the natural populations (SLA, stomatal density, foliar %N), or the snow removal experiment (stomatal size and flowering phenology, see below). However, plasticity in δ¹⁵N was counter to expectations based on natural populations. Consistent with expectations, there were also trends for enhanced water-use efficiency (less negative δ¹³C) and reduced leaf water content in the lower elevation garden, but these differences were not significant after Bonferroni correction. Two traits (SLA and height at flowering) also had significant random effect for maternal family by garden (genotype × environment) indicating genetic variation in plasticity.

Analysis of trait variation also revealed significant genetic clines associated with elevation of origin for life history traits (Table S5; Fig. 2). Irrespective of the transplant environment, high elevation families flowered earlier (elevation of origin: $z = 4.43$, $P < 0.0001$) at smaller sizes (elevation of origin: $F_{1,999} = 14.6$, $P = 0.0001$) and for a shorter duration ($z = 3.82$, $P < 0.0001$) than low-elevation families.

Snow removal experiment

In the subset of plots in which *B. stricta* flowered, manipulations advanced snow melt by 17.4 ± 0.92 days (mean ± SE; treatment: $P < 0.0001$; Table 4; Fig. 3a). Snow removal caused snow to melt

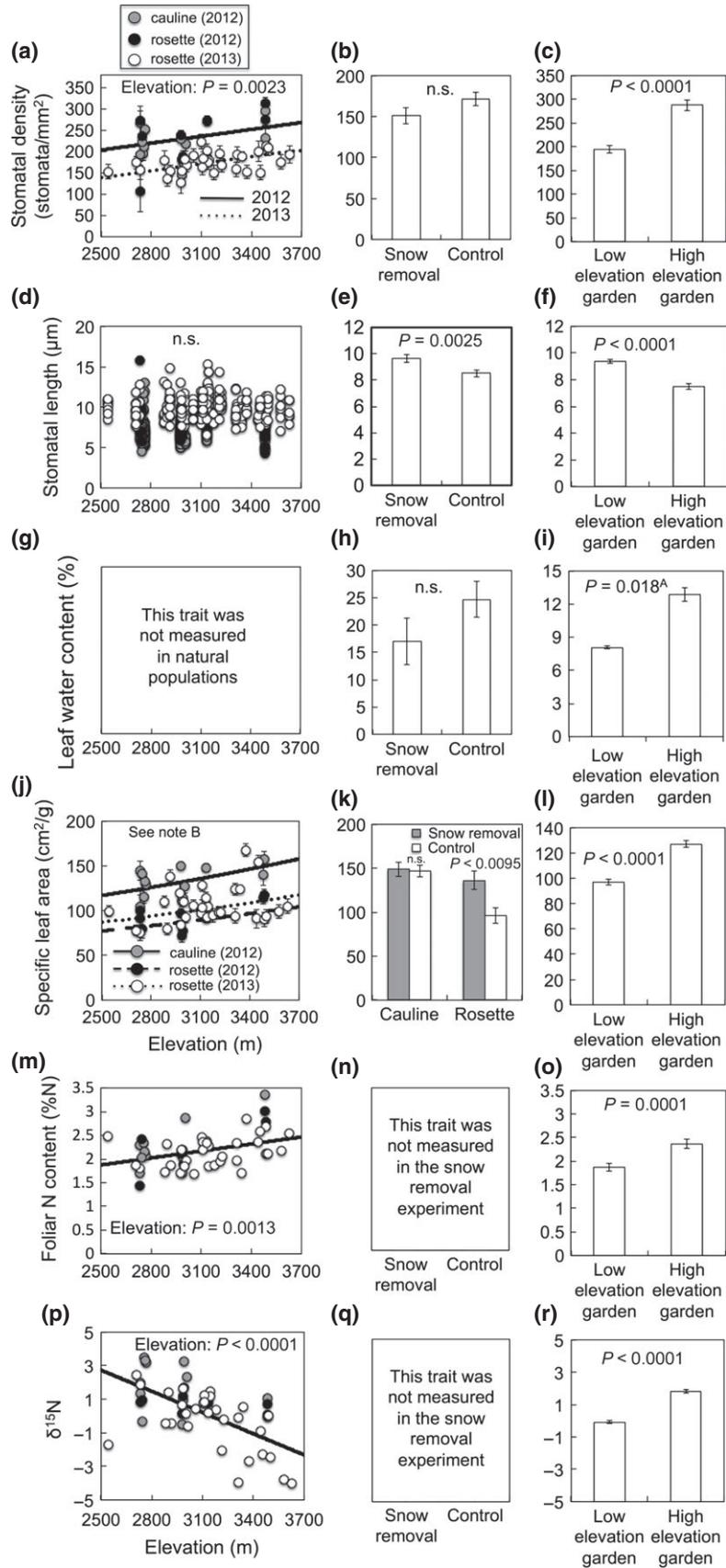


Fig. 1 Variation in foliar traits as a function of elevation in natural populations (left panels), snow removal manipulation (center panels), and common garden experiment (right panels). All data points have error (S.E.) bars, and n.s. indicates nonsignificant. For the natural populations, data points indicate the means (\pm SE) for cauline leaves (2012, gray circles), rosette leaves collected in 2012 (filled circles), rosette leaves collected in 2013 (open circles). Cauline leaves were not collected in 2013. There was no evidence for statistical interactions between elevation, leaf type, and year for any of the traits. In some cases, leaf type and/or year of collection influenced the foliar trait, and separate (parallel) regression lines are shown. Traits presented here are: stomatal density (panels a–c), stomatal size (d–f), leaf water content (g–i), specific leaf area (j–l), foliar N content (m–o), and $\delta^{15}\text{N}$ (p–r). We do not include panels for $\delta^{13}\text{C}$, as we found no significant clinal variation in this trait in natural populations or common gardens. Note (a) In univariate analysis, leaf water content varied plastically with garden (panel i), but the P -value (0.018) is higher than the Bonferroni corrected alpha (0.005). Note (b) Specific Leaf area was log transformed for analysis, but untransformed data are presented here, along with separate regression lines for the three classes of leaves because of significant effects of elevation ($P = 0.0077$), leaf type ($P < 0.0001$), and year ($P = 0.0069$).

earlier in all years; the treatment by year interaction was driven by a more pronounced difference between treatment and control plots in 2013 than the other years (Fig. 3a). A complementary analysis of all plots (even those that lacked *B. stricta* flowering individuals) showed that snow removal advanced snow melt by 15.4 ± 0.57 days ($P < 0.0001$; Table S6). Our soil moisture sensors failed, but a similar experiment in Grand Teton National Park (Wyoming) found that snow removed significantly reduces soil water content (Sherwood, 2013).

Previously, we correlated flowering phenology of *B. stricta* with snow melt records from 1973 to 2012 (Anderson *et al.*, 2012). According to those models, a 17.4 day advancement in the timing of snow melt would correspond with a 5.5 ± 1.3 to 10.3 ± 0.8 (mean \pm SE) day acceleration in first flowering and a 4.4 ± 2.1 to 9.4 ± 1.3 day acceleration in peak flowering (based on multivariate and univariate models presented in Table 1 of Anderson *et al.*, 2012). However, in that observational dataset, snow melt could have been correlated with some other factor that promoted early flowering.

Phenology. In this study, snow removal caused *B. stricta* to flower 7.05 ± 1.5 days earlier than when exposed to ambient snow melt (Fig. 3b, Table 4), consistent with expectations based on the long-term phenology dataset. The timing of first flowering of *B. stricta* advanced by 0.22 to 0.34 days/year from 1973 to 2012 (Anderson *et al.*, 2012); thus, snow removal accelerated flowering to the same extent as climate warming over 2–3 decades. Snow removal induced peak flowering to advance by 5.9 ± 1.4 days ($p = 0.0005$) and final flowering to advance by 6.1 ± 1.9 days ($P = 0.0058$, Table S7).

Finally, there was a significant relationship between the timings of first flowering and snow melt ($\beta = 0.33 \pm 0.07$ flowering days/snow melt days; $F_{1,8.73} = 21.1$, $P = 0.0014$; Fig. 3c), which nearly equaled the correlation from the long-term record (0.32 ± 0.07 flowering days/snow melt days in a model that exam-

ined flowering time as a function of year, snow melt date, and early spring temperature, see Anderson *et al.*, 2012). Site ($F_{2,13.8} = 9.98$, $P = 0.0021$) and year ($F_{2,14.3} = 8.08$, $P = 0.0045$) also influenced flowering time.

Foliar traits. Treatment ($F_{4,226} = 4.32$, $P = 0.0022$), site ($F_{4,88} = 5.81$, $P = 0.0003$), leaf type ($F_{4,226} = 7.61$, $P < 0.0001$), and the treatment by leaf type interaction ($F_{4,224} = 2.97$, $P = 0.020$) all significantly affected foliar traits in the multivariate analysis, but there was no effect of year ($F_{3,226} = 2.27$, $P = 0.08$). Univariate analyses revealed that snow removal induced higher specific leaf area (thinner leaves) for rosette leaves (treatment by leaf type: $F_{1,59} = 7.14$, $P = 0.0097$), and increased stomatal size irrespective of leaf type (treatment: $F_{1,57} = 10.0$, $P = 0.0025$; Table S8; Fig. 1). Treatment did not significantly influence leaf water content or stomatal density, although there was a marginal trend for the expected reduction in stomatal density in the snow removal treatment ($F_{1,59} = 3.35$, $P = 0.07$; Table S8). There was significant negative phenotypic correlation between stomatal density and stomatal size ($r = -0.32$, $P = 0.0019$, $N = 86$).

Discussion

Our studies in natural and experimental populations of *Boechera stricta* revealed extensive phenotypic plasticity in life history, foliar morphology, and ecophysiology in response to climate. As predicted, snow removal promoted early flowering, consistent with long-term positive correlations between the timing of snow melt and flowering phenology (Anderson *et al.*, 2012). Snow removal also altered foliar morphology in unexpected ways. The common garden experiment detected significant heritability in functional traits and plasticity, suggesting that populations could respond genetically to novel selection imposed on these traits by climate change. Clinal variation in traits from the natural populations was generally concordant with plasticity in the

Table 3 Broad-sense heritability (\pm SE) in traits and phenotypic plasticity in two common gardens. We estimated heritabilities from variance components in REML (Proc Mixed) with individual level data, used likelihood ratio tests (χ^2 , degrees of freedom = 1) to assess significance, and adjusted P -values for multiple tests with stepwise Bonferroni corrections (Proc Multtest). Significant heritabilities after stepwise Bonferroni correction are in bold

Trait	Low-elevation garden (2891 m)			High-elevation garden (3133 m)			Plasticity		
	H ²	χ^2	P -value	H ²	χ^2	P -value	H ²	χ^2	P -value
Day of first flowering	0.47 \pm 0.085	128.9	<0.0001	0.25 \pm 0.06	198.7	<0.0001	0.005 \pm 0.013	0.2	1.0
Duration of flowering	0.08 \pm 0.04	10.3	0.025	0.17 \pm 0.05	97.9	<0.0001	0.028 \pm 0.019	4.7	0.39
Height at flowering	0.29 \pm 0.08	56.7	<0.0001	0.49 \pm 0.08	482.3	<0.0001	0.052 \pm 0.025	15.1	0.0022
Specific leaf area	0.15 \pm 0.046	83.7	<0.0001	0.36 \pm 0.083	166.8	<0.0001	0.074 \pm 0.033	25.3	<0.0001
Stomatal density	0.12 \pm 0.06	10.0	0.028	0.035 \pm 0.06	0.4	1.0	0.06 \pm 0.06	1.8	1.0
Stomatal pore length	0.008 \pm 0.03	0.1	1.0	0.13 \pm 0.081	5.3	0.32	0	0	1.0
Leaf water content	0.01 \pm 0.01	1.6	1.0	0.026 \pm 0.014	13.6	0.0045	0.021 \pm 0.012	9.0	0.046
$\delta^{13}\text{C}$	0.20 \pm 0.11	4.9	0.38	0.49 \pm 0.12	13.8	0.0043	0.039 \pm 0.069	0.4	1.0
Foliar N content (%N)	0	0	1.0	0.34 \pm 0.14	6.3	0.19	0	0	1.0
$\delta^{15}\text{N}$	0	0	1.0	0.051 \pm 0.13	0.1	1.0	0.02 \pm 0.116	0	1.0

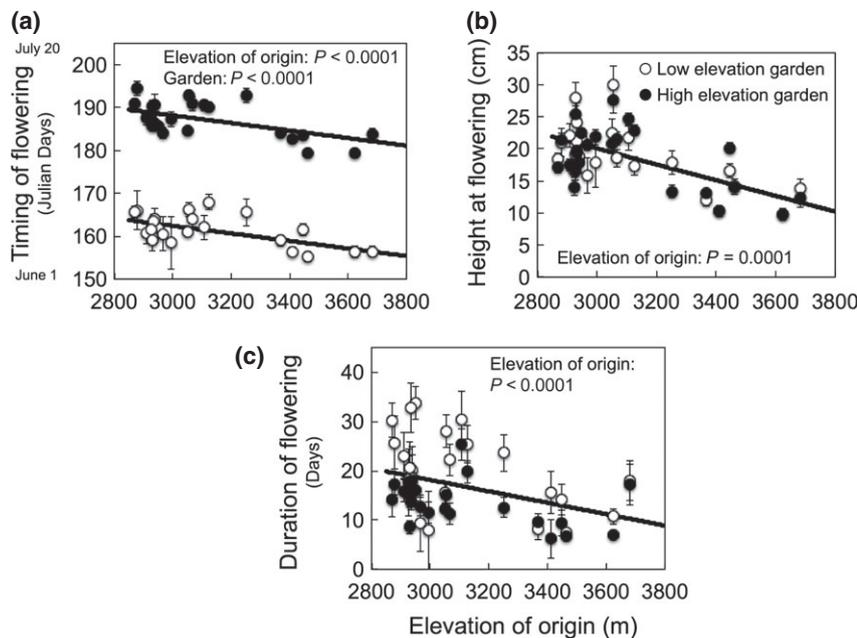


Fig. 2 In the common garden experiment, life history traits varied with elevation of origin, such that high-elevation genotypes flower earliest (a), at the smallest sizes (b) and for shorter periods of time than high-elevation genotypes (c). We conducted univariate analyses of traits using individual level data. Here, we show significant relationships between life history traits and provenance elevation using family level data, with SE bars for each family.

common gardens. Plasticity could allow *B. stricta*, and other species, to persist *in situ* despite changing climatic conditions.

Microenvironmental variation and phenotypic plasticity

In addition to broad associations between foliar phenotype and elevation, traits varied substantially within natural populations, which could reflect plas-

tic responses to microenvironment and/or extensive genetic variation. In the common garden experiment, we found significant effects of block nested within garden for 7 of 10 traits, indicating that small-scale environmental variation influences trait expression. Similar to other self-fertilizing species, *B. stricta* maintains limited within-population genetic variation (e.g. Song *et al.*, 2006); thus, we hypothesize that plasticity to the microenvironment could underlie

Table 4 The timing of snow melt and flowering varied as a function of treatment in the snow removal experiment. The residuals for flowering phenology were slightly heteroskedastic, so we implemented an unequal variance model with Satterthwaite approximation for degrees of freedom. The timing of peak flowering follows similar patterns (Table S6). Significant *P*-values are in bold

Source	Timing of snow melt		Timing of first flowering	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treatment	$F_{1,7.13} = 358.71$	<0.0001	$F_{1,20.3} = 22.77$	0.0001
Year	$F_{2,6.65} = 1627.93$	<0.0001	$F_{1,11.3} = 86.26$	<0.0001
Treatment × Year	$F_{2,5.77} = 8.71$	0.0181	$F_{2,12.4} = 0.84$	0.45
Site	$F_{2,13} = 0.61$	0.56	$F_{2,13} = 9.22$	0.0032

the considerable trait variation in natural populations.

Microtopography in alpine ecosystems can cause climatic conditions to differ as much locally as they do over hundreds of meters of elevation (Scherrer & Körner, 2010). Cool, moist conditions in local depressions and other topographic features could buffer natural populations from climate change, as propagules would not have to migrate as far to remain within the historical climatic regime (Scherrer & Körner, 2010; Dobrowski, 2011). For example, in the understories of temperate forests, microclimates created by the canopy are slowing the replacement of cold-adapted herbaceous plant species with warm-adapted species (De Frenne *et al.*, 2013). Could plasticity to microclimate allow populations to maintain positive growth rates and suffi-

cient genetic diversity long enough to adapt to novel conditions (Chevin *et al.*, 2010; Nicotra *et al.*, 2010)? Conversely, could climate change cause populations to contract into limited refuges, restricting population size, reducing population growth rates, and depleting genetic variation? Answering these open questions will reveal the true risk of extinction, especially for species with limited dispersal capacity. The challenge of addressing these issues will be to impose manipulations relevant to climate change in terrains with complex topography.

Flowering phenology

Snow removal induced a shift in flowering phenology equivalent to 2–3 decades of climate warming, which is

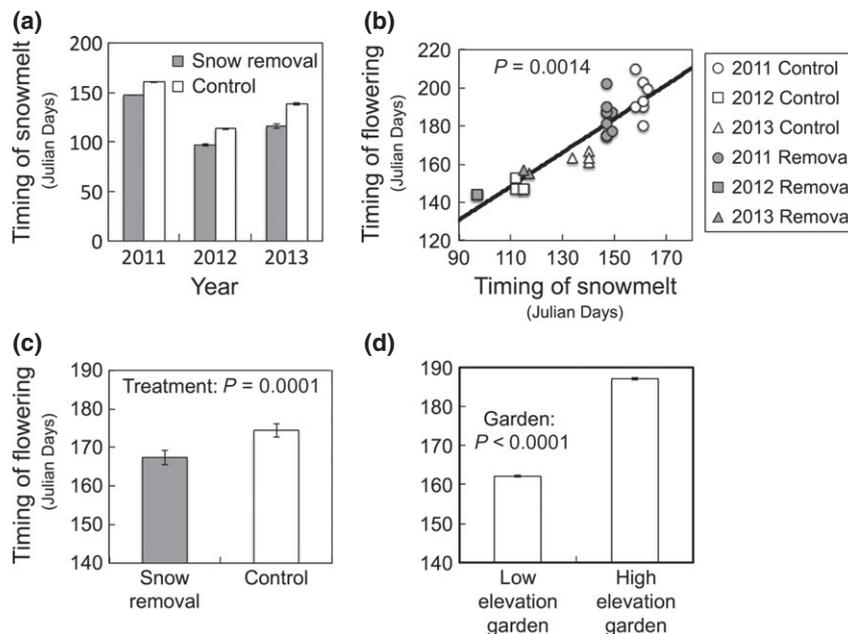


Fig. 3 Snow removal advanced the timing of (a) snow melt and (b and c) flowering phenology. (b) The positive correlation between snow melt timing and timing of flowering reflects long-term patterns in an observational dataset from 1973 to 2012 (Anderson *et al.*, 2012). (c) The treatment by year interaction was nonsignificant for flowering phenology; therefore, we have not separately plotted the effect of snow removal for each year. (d) Experimental transplants flowered significantly earlier in the low-elevation common garden than their siblings did in the high-elevation garden. Error bars are \pm 1SE.

similar to the effect of severe climatic events on flowering phenology in European systems (Jentsch *et al.*, 2009). In both common gardens, high-elevation genotypes flowered earlier at smaller sizes and set fruit more rapidly than low-elevation genotypes, which is likely an evolutionary response to short growing seasons at high elevations. In the future, later flowering immigrants from low-elevation populations could be at a disadvantage relative to local genotypes that flower earlier. However, other life history traits associated with high elevations, including small size at flowering and short duration of flowering, could reduce fitness under climate change. Climate change could impose selection for novel trait combinations, such as the rapid flowering of high-elevation genotypes and the long duration of flowering of low-elevation genotypes.

Longitudinal studies of natural populations and, to a lesser extent, snow manipulation experiments have extensively documented shifts in reproductive phenology in the context of climate change (e.g. Dunne *et al.*, 2003; Wipf & Rixen, 2010; Vedder *et al.*, 2013; Caradonna *et al.*, 2014). Plant functional traits also vary in response to climate, and strongly influence ecosystem-level processes and community dynamics (Lavorel & Garnier, 2002; Wright *et al.*, 2004; Adler *et al.*, 2014). Recently, Soudzilovskaia *et al.* (2013) concluded that functional traits, such as leaf water economy, can be used to predict changes in plant species abundance in response to increasing temperatures. Our snow removal provided the opportunity to document trait variation mediated by a key climatic agent of selection: snowbank dynamics. However, we cannot assess the degree to which plastic changes in plant traits reflect long-term shifts within populations, as long-term data on functional traits are exceptionally rare (Soudzilovskaia *et al.*, 2013). We therefore suggest that future longitudinal studies quantify functional traits to explore the evolutionary and ecosystem-level ramifications of climate change.

Foliar traits

Historically, the shorter growing seasons at higher elevations likely favored traits that enable rapid development, such as high stomatal density, thin leaves (high SLA), and increased foliar N content. Those traits would have been detrimental in lower sites prone to stress from drought and herbivores. We hypothesized that snow removal and subsequent soil drying would induce trait values resembling those of drier low-elevation populations. Our results did not broadly support the hypothesis.

In contrast with other studies (e.g. Scheepens *et al.*, 2010), we found that specific leaf area (SLA) increased

with elevation in natural populations and experimental gardens. This pattern could reflect increasing water availability and/or declining growing season length with elevation. Snow removal induced thin leaves (high SLA), which was unexpected based on clinal variation in SLA in this study. Our finding runs counter to observations that recent climate change has favored alpine species with lower SLA in the Caucasus mountains (Soudzilovskaia *et al.*, 2013). *Boechea stricta's* plasticity in SLA could be maladaptive. Alternatively, other environmental conditions that favor high SLA, such as low light, might covary with snow removal, or SLA could be genetically correlated with traits under selection.

We found substantial plasticity in stomatal anatomy. Stomatal conductance generally declines with increasing drought (e.g. Medeiros & Ward, 2013), yet few studies have examined variation in stomatal morphology in response to soil moisture (Woodward *et al.*, 2002; Casson & Gray, 2008; Zhou *et al.*, 2013) or snowbank dynamics. As expected (e.g. Mcelwain, 2004), stomatal density increased with elevation in our natural populations and experimental gardens, which could have resulted from declining partial pressure of CO₂ with elevation, or reduced water stress at higher elevations. Snow removal induced significantly larger stomata, and there was a marginal trend for decreased stomatal density. Increasing atmospheric (CO₂), decreasing soil water content and increasing temperatures associated with climate change could act in concert to favor reductions in stomatal density (Luomala *et al.*, 2005; Casson & Gray, 2008), enabling plasticity, or adaptation to keep pace with changing conditions.

In the common garden experiment, leaf water content was greater in the higher elevation environment. We found no difference in leaf water content as a function of treatment in the climatic manipulation, but our sampling was restricted to only 1 year (2013). We were unable to quantify plasticity in response to snow removal for ecophysiology due to instrument failure. We predict that warming temperatures and altered snow dynamics will disrupt plant water status, or induce enhanced water-use efficiency.

Most nitrogen in plant leaves occurs in proteins involved in photosynthesis; thus, foliar N content provides a reasonable proxy for photosynthetic capacity (Evans, 1989). Not surprisingly, foliar N content increased with elevation in both natural populations and common gardens. Enhanced foliar N content could be advantageous in the short growing seasons of high-elevation sites, where it would promote rapid development. In contrast, selection could favor reduced foliar N at low elevations if herbivore pressure is higher there, as lower foliar N diminishes the nutritional quality of

plant tissue for herbivores (e.g. Rasmann *et al.*, 2014). Indeed, resistance to insect herbivory declines with elevation of origin in *B. stricta*, which could influence the joint evolutionary trajectories of these traits (J.T. Anderson, N. Perera, B. Chowdhury & T. Mitchell-Olds, in review).

Our results from natural abundance stable N ratio are consistent with global patterns (Craine *et al.*, 2009): foliar $\delta^{15}\text{N}$ showed the highest values in the hot, dry lower elevation sites. Researchers have documented a positive correlation between foliar N content and foliar $\delta^{15}\text{N}$ in many ecosystems as soil N tends to be high in hot, dry areas (Craine *et al.*, 2009), yet we found no correlation between these traits in the natural populations. This lack of correlation could arise if soil N increases with elevation (in contrast with previous studies), or if high elevation plants are more efficient at taking up N from the soil. If herbivore populations grow as ecosystems warm, climate change could impose selection for reduced foliar N. However, as foliar N is tightly associated with soil N levels (e.g. Campbell *et al.*, 2010), plants may have limited potential to respond to novel selection on this trait mediated by climate change.

Our datasets cannot test whether trait plasticity will be favored by selection under future climates, or will be maladaptive, decreasing population growth rates and increasing the risk of extinction (Ghalambor *et al.*, 2007; Nicotra *et al.*, 2010). To do so, future studies will need to manipulate climatic agents of selection in quantitative genetic field studies, exposing siblings to contemporary conditions and simulated climate change, while quantifying fitness components and functional traits. Resulting datasets will allow analysis of whether trait plasticity confers a fitness advantage or cost under simulated climate change. If climate change imposes strong selection for phenotypic plasticity, then populations that harbor significant genetic variation in plasticity for key traits could evolve steeper reaction norms for those traits. Our ongoing field studies in *B. stricta* will address these issues as we have conducted snow removal experiments in five common gardens using replicated maternal families of known origin.

Boechera stricta maintains substantial plasticity in ecologically relevant traits, which could enable populations to track changing climates even in the absence of migration. Our experimental manipulation of snowpack accurately reflects long-term temporal changes in flowering phenology in this system (Anderson *et al.*, 2012), but we found novel trait combinations within the snow removal plots. Although stomatal anatomy responded as we predicted based on phenotypic clines, specific leaf area followed the opposite pattern. In contemporary environments, climatic conditions are changing rapidly, but other agents of selection remain

unaltered, such as photoperiod and bedrock. This decoupling of previously reliable environmental cues could favor new suites of trait values that do not simply represent phenotypes that were historically advantageous at lower elevations, or could induce maladaptive phenotypic shifts that have negative consequences for population persistence. It is still unclear whether plasticity in some traits could lead to eventual adaptation, or whether plastic variation in traits will be insufficient once climates change past some threshold levels. Plasticity could allow populations to withstand moderate climate change, providing more time for evolutionary rescue via gene flow (Carlson *et al.*, 2014). Specifically, introgression of warm- or dry-adapted alleles from lower elevation populations could result in local adaptation to novel climates. Taken together, our results suggest plasticity could play a prominent role in *B. stricta*'s response to climate change, but whether that role will promote or delay adaptive evolution remains to be investigated.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1–S3. Maps of control vs. snow removal plots in all three snow manipulation sites.

Table S1. Coordinates of natural populations, snow removal sites, and common gardens.

Table S2. Coordinates for populations of families in the common garden experiment.

Table S3. The timing of snow melt and flowering as a function of treatment based only on data from (a) 2011 when we monitored flowering phenology at all three sites, and (b) the Marriage Meadow site, where we collected data from 2011 to 2013.

Table S4. Phenotypic correlations between foliar traits in the natural populations.

Table S5. Effect of elevation of origin, transplant garden, and cohort on foliar and life history traits in univariate analyses.

Table S6. Repeated measures ANOVA of the timing of snow melt course of the experiment, including data from all plots, even those in which we found no flowering *B. stricta* individuals.

Table S7. Effects of experimental snow removal on the timing of peak flowering and last flowering (repeated measures ANOVAS).

Table S8. Univariate ANOVAS examining the effect of treatment (snow removal or control), leaf type (cauline vs. rosette), year, and interactions on *Boechera stricta* foliar traits (stomatal density and size, leaf water content, specific leaf area).